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**MECHANISM OF HYDROGEN PRODUCTION IN [Fe-Fe]-
HYDROGENASE: A DENSITY FUNCTIONAL THEORY
STUDY (PREPRINT)**

Steven Trohalaki and Ruth Pachter

**Hardened Materials Branch
Survivability and Sensor Materials Division**

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Mechanism of Hydrogen Production in [Fe-Fe]- Hydrogenase: A Density Functional Theory Study

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ABSTRACT

[Fe-Fe]-hydrogenases are a class of metalloenzymes that catalyze the production of H_2 from two protons and two electrons. In this work, we used density functional theory (DFT) calculations to analyze the mechanism of hydrogen production, providing insight into the role of the intermediates in the catalysis. We also validated the exchange-correlation functional applied within DFT for model compounds of the active site in [Fe-Fe]-hydrogenase, enabling us a reliable application for understanding previously established hydrogen production hypotheses, as well as providing a starting point for a future investigation of the effects of the protein environment on the catalytic mechanism of [Fe-Fe]-hydrogenases.

KEYWORDS: Hydrogenase; hydrogen; density functional theory

INTRODUCTION

Recently Prince et al.¹ assessed the potential efficiency of photobiological hydrogen production² as a renewable fuel, regarding the maximal energetic efficiency when using hydrogenases. It was noted that additional improvements in hydrogen generation at the biochemical level are required.² In our first step towards understanding the mechanism, we explored all-iron hydrogenases. The active site domains of the all-iron hydrogenases from the sulfate-reducing microorganism *Desulfovibrio desulfuricans* (DdH)³ and from the anaerobic soil microorganism *Clostridium pasteurianum* (CpI)^{4, 5} were found to be very similar,⁶ although it has been inferred from the manner in which the crystals were obtained that the CpI structure represents an oxidized state and that the DdH structure represents a reduced state,⁶ while Nicolet, et al.,⁷ suggested that the DdH crystal represents a mixture of anaerobically oxidized and reduced states. The crystal structures for active sites in the [Fe-Fe]- and [Ni-Fe]-hydrogenases have also been shown to be surprisingly congruent,^{7, 8} reflecting convergent evolution.⁹ However, although [Fe-Fe]-hydrogenases and [Ni-Fe]-hydrogenases readily catalyze the interconversion between the hydrogen molecule and two protons plus two electrons,¹⁰ limitations, such as the sensitivity to O₂,^{11, 12} still need to be resolved. Moreover, an understanding of the dependence of the protein environment on the catalytic mechanism is still lacking, as recently reviewed.⁹

In order to gain insight into the catalytic activity of [Fe-Fe]-hydrogenases, a number of theoretical density functional theory (DFT) studies emerged,¹³⁻²³ focusing mostly on the Fe₂S₂ subcluster in the active site, (abbreviated [2Fe]_H), while excluding the remaining subcluster in the active site, namely the Fe₄S₄ cubane (abbreviated [4Fe-4S]_H, which is linked to [2Fe]_H through a cysteinyl sulfur (see Figure 1). Considering both theory and experiment, Armstrong⁹ outlined the relationships between different states of [2Fe]_H, addressing structure, mechanisms of activation, and hydrogen cycling (see Figure 3 in reference 9) as well as posing several questions.⁹ In this work, we have modeled the intermediates in Armstrong's outline⁹ as a starting point for elucidation of the mechanism and expanded on it by including an additionally oxidized species. We endeavor here to confirm this outline and provide insight into the questions posed. We also validated the x-c functional to be applied within DFT for a

model system for $[\text{Fe}_2]_{\text{H}}$ and for two other model compounds for the Cpl active site, enabling us a reliable application. The structural changes we calculated for the different functionals emphasize the importance of applying the appropriate level of theory, and thus our work will serve as the starting point for future investigations of the effects of the protein environment on the catalytic mechanism of $[\text{Fe-Fe}]$ -hydrogenases.

METHODS

DFT calculations at the BP86^{24, 25}/LACV3P**²⁶ and B3LYP^{25,27, 28}/LACV3P**²⁶ levels were performed with the software package Jaguar.²⁹ Note that the two Fe atoms in $[\text{2Fe}]_{\text{H}}$ are termed proximal and distal (Fe_p and Fe_d), which refer to their proximity to the $[\text{4Fe-4S}]$ subcluster (we also use subscripts p and d to indicate to which iron atom ligands are coordinated). The coordination geometry of $[\text{2Fe}]_{\text{H}}$ with a carbonyl (CO_b) bridging the Fe atoms is that of two edge-bridged square pyramids; the CN, CO, and noncysteinyll sulfur atoms form the base and the axial position is occupied by the bridging carbonyl, CO_b . In some unbridged structures, CO_b rotates $\sim 180^\circ$ around Fe_d but still occupying an axial position, resulting in an inverted square pyramid about Fe_d , although the two square pyramids still share an edge. We used a dihedral angle, ϕ_{CO} , to characterize the orientation of CO_b in $[\text{2Fe}]_{\text{H}}$; ϕ_{CO} is defined as $\text{X-Fe}_d\text{-Fe}_p\text{-CO}_b$, where X is the middle atom in either the propane or dimethylamine that bridges the noncysteinyll sulfur atoms.

First, three model compounds of the active site were considered in the calculations (**A**, **B**, and **C** in Figure 2): **A** is $(\mu\text{-PDT})\text{Fe}_2(\text{CO})_3(\text{CN})_2(\text{CH}_3\text{S}^-)$ (**5** in Ref. 17); **B** is $[\text{Fe}_2]_{\text{H}}$ based on the Cpl x-ray structure⁴ with CH_3S^- substituted for cysteine and the Fe_4S_4 cubane removed; **C** is $[\text{Fe}_2]_{\text{H}}$ based on the Cpl x-ray structure⁴ coordinated to the Fe_4S_4 cubane through Cys503. Initial construction of model compounds **B**, **C**, and those in Armstrong's outline⁹ (model compounds **1-8**) used the coordinates for residues 580, 581, and 503, from the Cpl crystal structure,⁴ i.e., for $[\text{Fe}_2]_{\text{H}}$, the Fe_4S_4 cubane, and the cysteine, respectively. Hydrogen atoms were added as appropriate and the one of the two lone oxygen atoms in residue 580 was assumed to be a water molecule ligated to Fe_d . For **B**, **C**, and **1-3**, the

following modifications were made: 1) One carbonyl group coordinated to Fe_p and one coordinated to Fe_d were each changed to cyano groups, which is consistent with spectroscopic evidence³⁰⁻³² and with the presence of these groups ligated to the Fe atom in the active site of [Ni-Fe]-hydrogenase;³³ 2) The two sulfur atoms in $[\text{2Fe}]_H$ were bridged with dimethylamine ($-\text{CH}_2\text{-NH-CH}_2-$). (This bridge essentially replaced the second lone oxygen atom in the CpI crystal structure⁴ whose original assignment was made in lieu of unresolved atoms bridging the sulfur atoms.) This bridging moiety has been previously suggested³⁴ because it can better act as a base than propane, which was originally presumed. It is also probable that the bridging moiety is the same for both CpI and DdH.⁹ For **A**, however, we used propane as the bridging moiety so that we could compare our results to previous work.¹⁷ Note that we denote the noncysteinyll sulfur atoms in $[\text{2Fe}]_H$ with subscripts a or b signifying that they lie either above or below the plane defined by Fe_p , Fe_d , and the middle heavy atom in the bridging moiety.

For **4–8**, the following additional modifications were made: 3) Following Zhou, et al.,²² a CH_3SH group was coordinated to each of the three tri-coordinated Fe atoms in the Fe_4S_4 cubane. The CH_3SH groups might serve to provide an approximate protein environment for the Fe_4S_4 cubane as well as to prevent coordination between the Fe_4S_4 cubane and the cysteine, which is not observed in the crystal; 4) The nitrogen atom on the cysteine was capped by a hydrogen and the carbonyl was capped with an NH_2 , i.e., the nitrogen on the next residue in the sequence was included and capped with two hydrogens. The peptide bond between the cysteine and residue 504 is thereby maintained. Current model compounds could therefore be used in a quantum mechanical/molecular mechanical model and results could be compared in a straightforward manner. For **5**, the bridging carbonyl was rotated $\sim 180^\circ$ and the Fe-C-O bond angle changed to 180° . For **6–8**, one or two hydrogen atoms were added as shown in Figure 2.

For **A** and **B**, molecular charges of -1, -2, and -3 were employed to simulate the oxidized, semi-reduced, and reduced forms. For **C**, molecular charges of +1, 0, and -1 were employed because of the presence of the $[\text{4Fe4S}]_H$ subcluster, which was shown experimentally to have a +2 charge.¹⁹ Molecular charges for **1** and **2** are +1 and 0, respectively, and -1 for **3–8**. Spin states for the semi-reduced forms for **A**, **B**, **C**, **2**, and **6** are $\frac{1}{2}$; all others are 0. Geometry optimizations were performed using initial

coordinates as described above but, in addition, starting from optimal geometries for compounds lying adjacent in Armstrong's scheme (Figure 3 in reference ⁹). Geometry optimizations were performed with nitrogen in the dimethylamine bridging moiety oriented towards Fe_p and towards Fe_d. For open-shell systems both restricted-spin and unrestricted-spin calculations were performed. Geometries reported here correspond to the lowest lying energy minima we found, except when the conformations differ substantially from the crystal structure, i.e., those structures that display an RMS greater than 1 Å. In some cases, we found isoenergetic optimal conformations.

RESULTS AND DISCUSSION

In previous calculations of model compounds of the [2Fe]_H, applications ranging from the gradient generalized approximation (GGA) exchange-correlation (x-c) functional within DFT e.g., Perdew-Wang,^{21, 22} BP86,³⁵ to the hybrid B3LYP x-c functional¹⁸ were used. In a study for a related model compound, it was attested recently that B3LYP would result in poorer agreement with experimental structures,²⁰ even though that group applied B3LYP in earlier work.¹⁵⁻¹⁷ The geometry of the reduced form of **A** (Table 1) optimized at B3LYP/LACV3P** shows that a carbonyl group (CO_b) partially bridges the Fe_p and Fe_d atoms (see Figure 3), which is consistent with previous work,¹⁷ and with the asymmetrical bridging inferred in the crystal structure of [2Fe]_H in DdH (Note: the initial assignment of an oxygen atom or water molecule as the bridging ligand for [2Fe]_H in DdH was due to a mixture of reduced and oxidized states leading to the unresolved carbon atom.⁷) However, because it has been recently suggested that a non-hybrid GGA x-c functional may be more appropriate,²⁰ we also optimized geometries at BP86/LACV3P**, which effects distinct changes; in particular, a more tightly bridged structure results (see Figure 3 and Table 1), which is inconsistent with B3LYP-optimized geometries (either with ours or with the results of Bruschi, et al.¹⁷) or with the asymmetrical bridging found in the crystal structure of [2Fe]_H in DdH.⁶ The Fe_p-Fe_d distance we find for the reduced state of **A** is very close to the value observed for CpI, even though this crystal structure is thought to be an oxidized state. The optimal molecular geometry obtained for fully reduced **A** by Cao, et al.,¹⁸ who employed B3LYP

with a mixed basis set, displays no carbonyl bridging Fe_p and Fe_d . Interestingly, the optimal geometry for a compound similar to **A** (but with a CH_3SH ligand instead of CH_3S^- and, therefore, a -2 charge instead of -3) reported by Zhou et al.,²¹ who used PW92³⁶ with a basis set of double-zeta quality, has values for $\text{Fe}_d\text{-CO}_b$ and $\text{Fe}_p\text{-CO}_b$ distances of 1.746 and 2.921 Å. Indeed, although Zhou, et al.,²² have also extended their calculations to include the $[\text{4Fe-4S}]_H$ subcluster, it is difficult to assess the accuracy of their results because they were obtained with a smaller basis set and the PW x-c functional. The hybrid x-c functional PBE0³⁷ yields an optimal geometry more similar to the semi-bridged B3LYP structure than to the tightly bridged BP86 conformation. The sensitivity of the x-c functionals is demonstrated by comparison of the partial atomic charges calculated using a Natural Bond Order³⁸ population analysis (see Table 2).

The oxidized and semi-reduced forms of **A** do not fit into Armstrong's scheme⁹ and are only of interest in terms of a biomimetic system because **A** lacks a water ligand, as observed in the CpI crystal structure. For semi-reduced **A**, we obtained bridged and unbridged optimal conformations, in agreement with Bruschi, et al.¹⁷ For B3LYP and PBE0, the differences in energy are about 4.5 kcal/mol; for BP86, the difference is about 2 kcal/mol. Geometrical parameters are presented in Table 3 and compare reasonably well with the CpI crystal structure. The most notable difference is that the $\text{Fe}_p\text{-Fe}_d$ distances in the unbridged conformations obtained with B3LYP and PBE0 are 0.17 Å and 0.9 Å longer than observed in the CpI crystal structure. The molecular geometries we obtained with restricted-spin calculations are very similar to the unrestricted-spin results in Table 3 but the restricted-spin geometries are 6.6 and 7.6 kcal/mol higher in energy than the unrestricted-spin B3LYP- and PBE0-optimized structures, respectively. For all three functionals, $\langle S^2 \rangle$ values for the bridged conformations are about 0.8, in good agreement with the value expected for one unpaired electron, indicating only minor spin contamination. However, $\langle S^2 \rangle$ values are 1.6, for the unbridged conformations obtained with B3LYP and with PBE0, which indicates additional spin contamination (one would expect $\langle S^2 \rangle$ to be 3.75 for additional unpaired electrons), although for BP86, $\langle S^2 \rangle$ for both the bridged and unbridged structures is less than 0.8.

BP86 geometry optimizations for the oxidized form of **A** (Table 4), when starting with both bridged and unbridged geometries, resulted only in bridged structures, while applying B3LYP and PBE0 yielded both bridged and unbridged structures with the bridged structure lower in energy by 0.81 and 4.2 kcal/mol, respectively, for the two functionals. The unbridged B3LYP and PBE0 structures have large Fe_p-Fe_d separations – 3.198 and 3.078 Å, respectively – although the axial carbonyl is rotated to almost the same degree as in the bridged structures, i.e., $\phi_{\text{CO}}=173^\circ$. In contrast, Bruschi, et al.,¹⁷ employing B3LYP, found only a bridged conformation for oxidized **A**. It appears that B3LYP/LACV3P** level of theory is able to locate the two local minima on the potential energy surface for the oxidized form of **A** corresponding to the bridged and unbridged conformations, while B3LYP with the mixed basis set used by Bruschi, et al.,¹⁷ or BP86/LACV3P** are not. It is also notable that the Fe_p-CO_b distance is shorter than Fe_d-CO_b in the bridged structures we obtained with B3LYP, PBE0, and BP86, in agreement with the B3LYP-optimized geometries obtained by Bruschi, et al.¹⁷

Model compound **B** with molecular charges of -1, -2, and -3 and model compound **C** with molecular charges of +1, 0, and -1 are analogous to the first two compounds in Armstrong's scheme⁹ and one additionally oxidized form, i.e., compounds **1**, **2** and **3**. Molecular geometries optimized at B3LYP/LACV3P** were all found to display a carbonyl (CO_b) bridging Fe_p and Fe_d, in agreement with Armstrong.⁹ It appears that the water ligand in **B** and **C** precludes the unbridged structures observed for **A**. Geometrical parameters agree with the crystal structures (see supplemental material) with two exceptions. For the oxidized form of **B**, the separation between Fe_d and the water ligand is 0.9 Å shorter than in the CpI crystal structure. However, the first reduction increases Fe_d-water separation by 0.14 Å and the second reduction increases it by an additional 0.3 Å, which is consistent with the departure of the water ligand from the active site upon reduction. For **C**, Fe_d-water separation is longer than that in the CpI crystal structure by 0.9 Å for all three charge states. In addition, compound **C** displays a close contact between the carbonyl oxygen in the cysteine and an Fe atom in the [4Fe-4S]_H subcluster. This contact, which is not observed in the crystal structures, results from deformation of dihedral angles from the values observed in the crystal and is prevented from occurring in model compounds **1-8** by the

CH₃SH groups appended to [4Fe-4S]_H. The frontier molecular orbitals for the three charge states of C (see Figure 4) are localized on both the [4Fe-4S]_H and [2Fe]_H subclusters as well as on the sulfur bridge. In addition, the total spin density for semi-reduced C resides mostly on [4Fe-4S]_H and only partially on [2Fe]_H (see Figure 4), in contrast to results reported by Zhou, et al.,²¹ who found that the total spin density resides on [2Fe]_H for a model compound that excludes [4Fe-4S]_H. It is therefore obvious that the [4Fe-4S]_H subcluster must be included in model compounds for the [Fe-Fe]-hydrogenase active site if further mechanistic details are to be discerned.

Geometrical parameters for compounds 1-8 are presented in Table 5. Agreement between the crystal structures and the optimal geometries is good except that the distance between Fe_d and the water ligand in the CpI crystal structure is 0.8 Å longer than in 1-3, which can be explained by other forces in the crystal acting on the water molecule. Our results agree with those reported by Schwab, et al.,³⁹ who reported for [2Fe]_H an Fe_p-Fe_d separation of 2.55 Å and Fe-S distances in the range of 2.33-2.35 Å for a structure composed of [4Fe-4S]_H and [2Fe]_H (but with a propane bridging the sulfurs in [2Fe]_H) and optimized with BP86 and a triple-zeta quality basis set. Optimal conformations for 1-8 are shown in Figure 5 in the same relation to each other as outlined by Armstrong.⁹ However, we found for several species other conformations that were nearly isoenergetic or only slightly higher in energy than the ones displayed in Figure 5; for 3, 4 and 5, conformations with the dimethyl amine bridge flipped toward Fe_d are only 0.2, 4.6, and 0.2 kcal/mol higher in energy, respectively. For the most part, Figure 5 follows Armstrong's scheme but the exceptions are notable. The optimal conformation we found for 2 (and that also displayed a small RMS difference with the CpI crystal structure) shows a carbonyl bridging Fe_p and Fe_d but the Fe_p-CO_b distance (1.833 Å) is shorter than the Fe_d-CO_b distance (2.457 Å). An optimal conformation 16 kcal/mol higher in energy has both Fe-CO_b distances almost equal, about 2.0 Å. We found that the equilibrium between 4 and 5 is not between bridged and unbridged conformations (as displayed in Figure 3 in reference 9); 4 is semi-bridged, with a Fe_p-CO_b distance of 2.800 Å and ϕ_{CO} equal to 173°, while in 5, the CO_b group has rotated away (ϕ_{CO} =134°) resulting in a Fe_p-CO distance 3.750 Å. However, the distance between Fe_d and the carbonyl coordinated primarily to Fe_p is 2.654 Å

and the dihedral corresponding to ϕ_{CO} ($\phi_{\text{CO}}' = \text{NH-Fe}_p\text{-Fe}_d\text{-CO}$) is 176° , so that **5** can also be considered to be in a semi-bridged conformation but with Fe_p and Fe_d bridged by a different carbonyl – the one coordinated only to Fe_p in **4**. Model compound **5** is lower in energy than **4** by 12.4 kcal/mol.

The question of whether the organic ligand bridging the sulfurs in $[\text{2Fe}]_{\text{H}}$, here dimethylamine, acts as a base in the addition of a hydrogen atom to model compound **5** was posed by Armstrong.⁹ Although we did indeed find an optimal conformation for model compound **6** in which the dimethylamino group is protonated, it is 65 kcal/mol higher in energy than the lowest-energy conformation we found. The energetically preferred location of the first added hydrogen atom in Armstrong's scheme⁹ is the nitrogen of the cyano group coordinated to Fe_d (see Figure 5). We also found for model compound **6** an optimal conformation with a hydride bridging Fe_p and Fe_d but this, too, is about 65 kcal/mol higher in energy than the lowest energy conformation. For model compound **7**, formed when a second hydrogen atom is added, we considered all combinations of two hydrogen atoms attached to the cyano ligands (to both the carbons and the nitrogens), to the nitrogen in the dimethylamino bridge, and to Fe_p and Fe_d via a hydride bridge. As shown in Figure 5, the lowest energy conformation, labeled **7a**, has a hydride bridge and a hydrogen atom bonded to the nitrogen of the cyano ligand coordinated to Fe_p . A conformation 9.2 kcal/mol higher in energy has a hydride bridge and a hydrogen atom bonded to the nitrogen of the dimethylamino bridge, although it is unclear how this conformation is involved in the formation of molecular hydrogen. Another higher-energy conformation that does appear to be involved in H_2 formation is labeled **7b** in Figure 5; it is similar to model compound **7a** except that the hydrogen originally bonded to the nitrogen atom in the cyano ligand coordinated to Fe_p has migrated to the cyano ligand's carbon atom and is separated from the hydride by only 1.7 Å. Model compound **7b** is 20.4 kcal/mol higher in energy than **7a** but only 2.3 kcal/mol lower in energy than model compound **8**, which has H_2 coordinated to Fe_p . A conformation 2.6 kcal/mol higher in energy than **8** has the NH in the dimethyl amine flipped towards Fe_d . Zhou, et al.,²² using the same model compound, obtained a structure similar to **7b** but only for the oxidized and semi-reduced states, i.e., with charges of +1 and 0, and did not find an optimal geometry with H_2 coordinated to Fe_p , as in our model compound **8**, for any

charge state. Superimposing either model compound **7b** or **8** with the CpI crystal structure for $[2\text{Fe}]_{\text{H}}$, $[4\text{Fe-4S}]_{\text{H}}$, and the cysteine linking them, we found RMS differences of 0.74 Å and 0.66 Å, respectively. (For this calculation, we changed two of the CO groups in the CpI structure to CN and we included only atoms that were common to both the crystal structure and the model compounds. In addition, we did not include the bridging CO in the crystal structure and the corresponding CO in the model compounds, which is rotated $\sim 180^\circ$.) Superposition of **8** with the CpI crystal structure⁴ is shown in Figure 6. The largest difference is in the shape and orientation of the $[4\text{Fe-4S}]_{\text{H}}$ subcluster, which is much closer to a perfect cube in **8** than in the crystal structure. The difference in orientation is due to coordination between the cysteine's carbonyl group and one of the CH_3SH groups.

Plots of the highest occupied and lowest unoccupied molecular orbitals for **7b** and **8** (see Figure 7) are similar for the two species; the HOMO is localized on the $[4\text{Fe-4S}]_{\text{H}}$ and the LUMO on the $[2\text{Fe}]_{\text{H}}$. Using NBO analysis,³⁸ we found that for model compound **7b** the partial charge on the hydride bridging Fe_p and Fe_d is -0.06 while the partial charge on the hydrogen bound to CN_p is +0.27. For model compound **8**, the partial charge on the hydrogen bound to CN_p is +0.21, which is similar to model compound **7b**, and the partial charge for the other hydrogen atom in H_2 is +0.04. We removed the H_2 from model compound **8** and re-optimized the geometry, hoping to re-obtain model compound **5**. The resulting geometry was very close to model compound **5** except for a closer than usual contact (3.734 Å) between a sulfur in $[2\text{Fe}]_{\text{H}}$ and the Fe atom in $[4\text{Fe-4S}]_{\text{H}}$ that is coordinated to the cysteinyl sulfur. Imposing our requirement that conformations must be reasonably close to the crystal structure, we rejected this conformation but do not believe that it is evidence that model compounds **8** and **5** do not represent species that are in equilibrium.

CONCLUSIONS

By comparing calculated to experimental structures for $[2\text{Fe}]_{\text{H}}$, we determined an appropriate level of theory for investigating the mechanism of hydrogen production in $[\text{Fe-Fe}]$ -hydrogenase. In addition, we showed that model compounds for the $[\text{Fe-Fe}]$ -hydrogenase active site should include the $[4\text{Fe-4S}]_{\text{H}}$

cluster in addition to $[2\text{Fe}]_{\text{H}}$, although almost all previous theoretical studies include only $[2\text{Fe}]_{\text{H}}$. We also showed that optimal geometries for some of the intermediates in a previous hypothesis by Armstrong⁹ are somewhat different than previously thought, while for others, in particular fully reduced species with transient hydrogen species, ours is the first theoretical confirmation. We have established a starting point for a future investigation of the effects of the protein environment on the catalytic mechanism of $[\text{Fe-Fe}]$ -hydrogenases.

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Table 1. Geometrical Parameters for the Reduced Form of Model Compound A, a Compound Similar to A,²¹ and the Crystal Structure DdH³

Geometrical Parameter	B3LYP	BP86	PBE0	Bruschi, et al. ¹⁷	Cao, et al. ¹⁸	Zhou, et al. ²¹	DdH ³
Fe-Fe	2.612 Å	2.594 Å	2.543 Å	2.655 Å	2.655 Å	2.571 Å	2.566 Å
Fe _p -CN _p	1.956	1.903	1.923	1.939	1.941	1.88	1.845
Fe _p -CO _p	1.748	1.720	1.727	1.733	1.730	1.74	1.712
Fe _p -SCH ₃	2.451	2.440	2.382	2.406	2.415	2.19	2.505
Fe _d -CN _d	1.947	1.899	1.911	1.933	1.934	1.88	1.865
Fe _d -CO _d	1.742	1.714	1.717	1.725	1.727	1.74	1.797
Fe _d -CO _b	1.758	1.802	1.740	1.754	1.794	1.746	2.494 ^c
Fe _p -CO _b	2.730	2.181	2.559	2.638	—	2.921	2.807 ^c
Fe _p -S _a	2.384	2.396	2.346	2.387	—	2.28	2.304
Fe _p -S _b	2.402	2.410	2.355	2.396	2.400	2.28	2.290
Fe _d -S _a	2.408	2.384	2.353	2.404	2.403	2.28	2.279
Fe _d -S _b	2.400	2.346	2.351	2.390	—	2.28	2.298
Fe _d -C-O	167°	149°	165°	~164°	—	—	—
φ _{CO} ^a	-156	-171	-159	-157 ^b	—	—	174° ^c

^aφ_{CO} is defined in the text.

^bAs specified by Bruschi, et al.,¹⁷ this value was measured as the rotation of the CO group with respect to the plane defined by Fe_p, Fe_d, and the midpoint between S_a and S_b, which we've found to be nearly identical to our definition.

^cThe oxygen atom originally assigned as the bridging moiety is the DdH crystal structure was used in lieu of CO_b. Note: the initial assignment of an oxygen atom or (water molecule) as the bridging ligand for [2Fe]_H in DdH was due to a mixture of reduced and oxidized states leading to the unresolved carbon atom.⁷

Table 2. Partial Charges from a Natural Bond Order³⁸ Population Analysis for the Reduced Form of A
Calculated with Different Functionals

	Bruschi, et al. ¹⁷	B3LYP	BP86	PBE0
Fe _p	-0.022	0.0045	-0.276	-0.126
Fe _d	-0.249	-0.263	-0.343	-0.352
SCH ₃	-0.530	-0.613	-0.548	-0.575
S ₂ C ₃ H ₆	-0.612	-0.689	-0.525	-0.601
CO _b	-0.208	-0.145	-0.154	-0.137
CO _d	-0.204	-0.153	-0.103	-0.128
CN _d	-0.565	-0.565	-0.536	-0.549
CO _p	-0.019	0.0200	0.0189	0.0487
CN _p	-0.591	-0.601	-0.535	-0.582

Table 3. Geometrical Parameters for the Semi-Reduced Form of A and the Crystal Structure CpI⁴.

	Bridged				Unbridged				
Geometrical Parameter	B3LYP	BP86	PBE0	Bruschi, et al. ¹⁷	B3LYP	BP86	PBE0	Bruschi, et al. ¹⁷	CpI ⁴
Fe-Fe	2.592	2.563	2.532	2.597 Å	2.791 Å	2.538 Å	2.706 Å	2.587 Å	2.617 Å
Fe _p -CN _p	1.943	1.903	1.911	1.927	1.985	1.924	1.960	1.958	1.857 ^b
Fe _p -CO _p	1.766	1.740	1.744	1.768	1.779	1.741	1.759	1.757	1.754
Fe _p -SCH ₃	2.389	2.370	2.341	2.337	2.402	2.288	2.355	2.335	2.378
Fe _d -CN _d	1.946	1.912	1.919	1.916	1.943	1.909	1.912	1.930	1.863 ^b
Fe _d -CO _d	1.766	1.740	1.744	1.772	1.786	1.754	1.763	1.770	1.831
Fe _d -CO _{b/axial}	1.930	1.926	1.927	1.878	1.793	1.747	1.770	1.750	2.043
Fe _p -CO _b	2.082	1.976	2.003	2.116	—	—	—	—	2.100
Fe _p -S _a	2.405	1.363	2.357	2.379	2.418	2.324	2.383	2.372	2.319
Fe _p -S _b	2.410	2.388	2.360	2.386	2.354	2.346	2.314	2.355	2.340
Fe _d -S _a	2.380	2.348	2.336	2.375	2.361	2.304	2.314	2.312	2.337
Fe _d -S _b	2.363	2.321	2.319	2.371	2.376	2.323	2.331	2.355	2.320
Fe _d -C _b -O _b	142°	138°	140°		—	—	—	—	138°
φ _{CO} ^a	172	173	173		-9°	-13°	-9°	—	178° ^c

^aφ is defined in the text.

^bThe CN ligands are assigned as CO ligands in the CpI crystal structure.

^cFor the CpI active site, the midpoint between the two sulfur atoms was used to define φ_{CO}.

Table 4. Geometrical Parameters for the Oxidized Form of A and the Crystal Structure CpI⁴

Geometrical Parameter	Bridged				Unbridged		CpI ⁴
	B3LYP	BP86	PBE0	Bruschi, et al. ¹⁷	B3LYP	PBE0	
Fe-Fe	2.519 Å	2.507 Å	2.468 Å	2.516 Å	3.198 Å	3.078 Å	2.617 Å
Fe _p -CN _p	1.936	1.902	1.906	1.923	1.932	1.908	1.857 ^b
Fe _p -CO _p	1.802	1.769	1.776	1.789	1.768	1.749	1.754
Fe _p -SCH ₃	2.325	2.344	2.287	2.312	2.297	2.263	2.378
Fe _d -CN _d	1.927	1.881	1.902	1.914	1.927	1.901	1.863 ^b
Fe _d -CO _d	1.811	1.762	1.786	1.797	1.818	1.790	1.831
Fe _d -CO _{b/axial}	2.053	2.056	2.046	2.004	1.774	1.756	2.043
Fe _p -CO _b	1.911	1.857	1.866	1.929	3.315	3.193	2.100
Fe _p -S _a	2.370	2.353	2.324	2.367	2.355	2.299	2.319
Fe _p -S _b	2.371	2.333	2.325	2.352	2.402	2.337	2.340
Fe _d -S _a	2.335	2.274	2.298	2.338	2.362	2.322	2.337
Fe _d -S _b	2.333	2.213	2.298	2.339	2.384	2.349	2.320
Fe _d -C _b -O _b	134°	128°	133°	—	177°	177°	138°
φ _{CO} ^a	174	172	175	—	173	173	178° ^c

^aφ_{CO} is defined in the text.^bThe CN ligands are assigned as CO ligands in the CpI crystal structure.⁴^cFor the CpI active site, the midpoint between the two sulfur atoms was used to define φ_{CO}.

Table 5a. Geometry Parameters for Compounds 1-6

Bond	CpI ⁴	1	2	3	4	5	6
Fe-Fe	2.617	2.592	2.710	2.552	2.756	2.651	2.565
Fe _p -CN _p	1.857 ^b	1.933	1.926	1.934	1.933	1.917	1.954
Fe _p -CO _p	1.754	1.800	1.806	1.795	1.788	1.766	1.806
Fe _p -S _{cys}	2.378	2.312	2.423	2.345	2.309	2.191	2.425
Fe _d -CN _d	1.863 ^b	1.929	1.954	1.945	1.912	1.940	1.815
Fe _d -CO _d	1.831	1.842	1.821	1.817	1.814	1.814	1.800
Fe _d -CO _{b/axial}	2.043	1.869	2.457	1.932	1.774	1.761	1.804
Fe _d -OH ₂	2.959	2.125	2.192	2.112	—	—	—
Fe _p -CO _b	2.100	2.273	1.833	2.098	2.800	3.750	—
Fe _p -S _a	2.319	2.332	2.394	2.351	2.335	2.367	2.381
Fe _p -S _b	2.340	2.346	2.416	2.343	2.322	2.367	2.346
Fe _d -S _a	2.337	2.381	2.335	2.357	2.347	2.281	2.350
Fe _d -S _b	2.320	2.365	2.341	2.349	2.360	2.291	2.362
S _{cys} -Fe' _{cubane}	2.321	2.176	2.372	2.286	2.232	2.273	2.425
Fe _d -C _b -O _{b/axial}	138°	155°	121°	146°	172°	178°	178°
φ _{CO} ^a	178° ^c	174	-176	172	173	6° ^d	17

^aφ_{CO} is defined in the text.

^bThe CN ligands are assigned as CO ligands in the CpI crystal structure.

^cFor the CpI active site,⁴ the midpoint between the two sulfur atoms was used to define φ_{CO}.

^dThe dihedral N-Fe_p-Fe_d-CO_p is 176°.

Table 5b. Geometry Parameters for Model Compounds **7**, **8**, and for Two Similar Model Compounds due to Zhou, et al.²²

Bond	7a	7b	8	1a[1] _{ox} ^b	1a[0] _s ^b
Fe-Fe	2.578	2.595	2.672	2.564	2.577
Fe _p -CN _p	1.820	2.029	1.932	1.926	1.912
Fe _p -CO _p	1.800	1.817	1.812	1.768	1.765
Fe _p -S _{cys}	2.357	2.387	2.380	2.269	2.283
Fe _d -CN _d	1.931	1.934	1.935	1.864	1.870
Fe _d -CO _d	1.788	1.797	1.813	1.779	1.768
Fe _d -CO _{axial}	1.786	1.785	1.762	1.764	1.756
Fe _p -S _a	2.383	2.336	2.387	2.263	2.278
Fe _p -S _b	2.396	2.393	2.423	2.284	2.294
Fe _d -S _a	2.357	2.338	2.310	2.243	2.249
Fe _d -S _b	2.359	2.352	2.329	2.252	2.260
S _{cys} -Fe' _{cubane}	2.325	2.327	2.317	2.158	2.165
Fe _p -H'	—	1.617	1.574	1.635	1.599
Fe _p -H''	1.630	1.633	1.687	1.642	1.633
Fe _d -H''	1.656	1.678	1.902	1.681	1.681
H'-H''	4.180	1.699	0.882	1.713	1.685
NC-H'	—	1.179	1.874	1.207	1.234
φ _{CO} ^a	11°	11°	12°	10°	10°

^aφ_{CO} is defined in the text.

^bNotation used for the model compounds in the last two columns is the same as in reference.²² 1a[1]_{ox} and 1a[0]_s are oxidized and semi-reduced and have charges of +1 and 0, respectively.

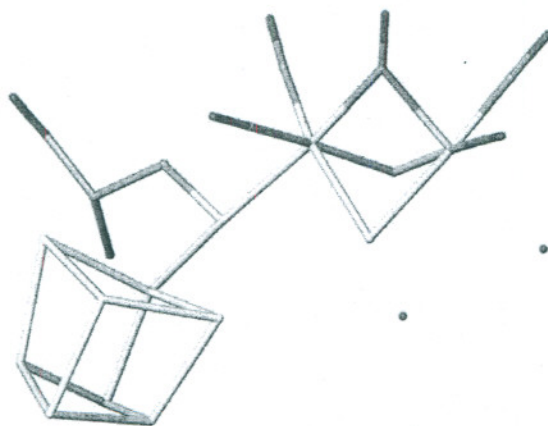
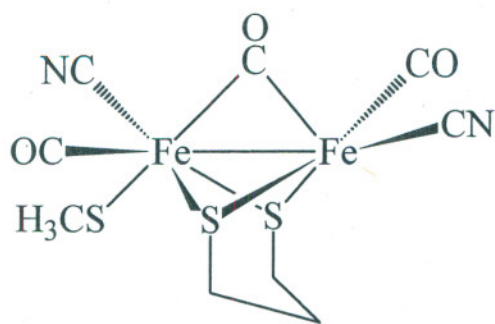
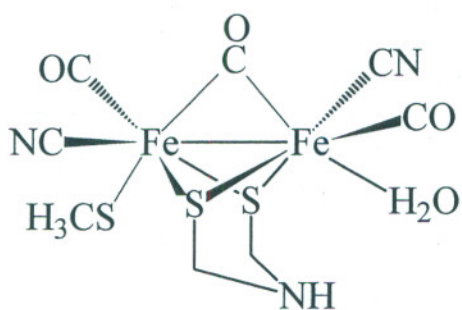


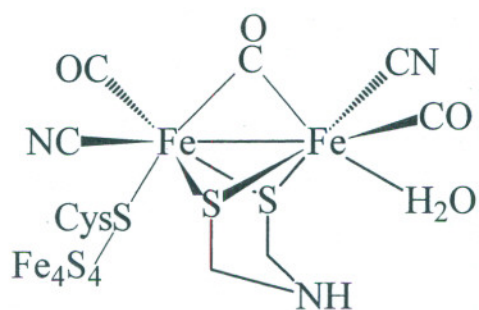
Figure 1. The [Fe-Fe]-hydrogenase active site from the CpI crystal structure , which consists of an Fe_2S_2 subcluster, termed $[\text{2Fe}]_{\text{H}}$, linked to a $[\text{4Fe-4S}]_{\text{H}}$ subcluster through a cysteinyl sulfur. Here, the cysteine is included. Red: oxygen; yellow: sulfur; blue: nitrogen; aqua: iron; and grey: carbon.



A



B



C

Figure 2. Model Compounds A, B, and C.

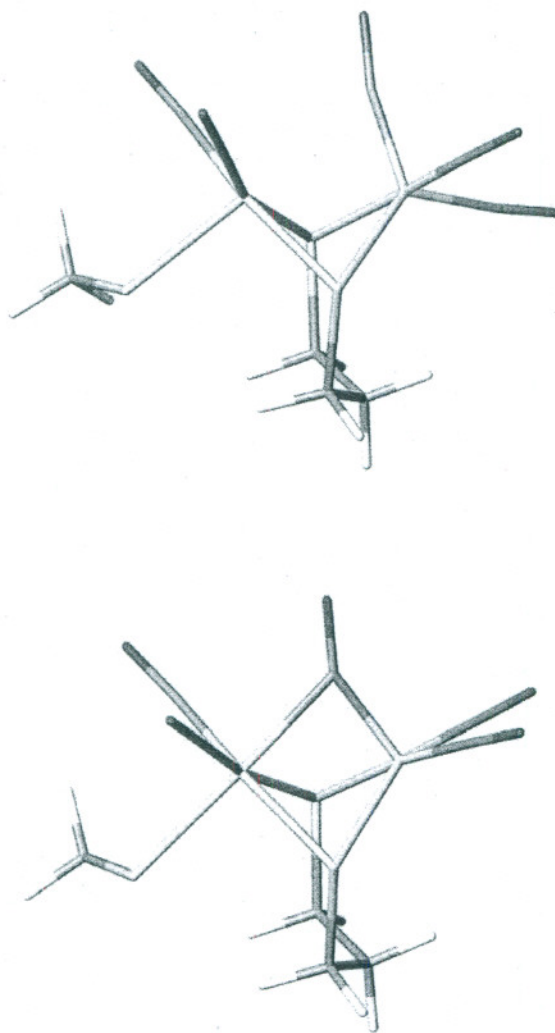
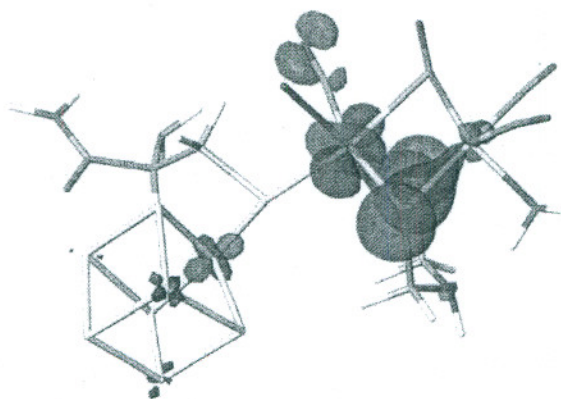


Figure 3. Minimum energy conformations for the reduced form of A. Conformations for model compound A optimized at B3LYP/LACV3P** (a) and at BP86/LACV3P** (b). Conformation (b) is more tightly bridged with a $\text{Fe}_p\text{-CO}$ distance of 2.181 Å, compared to 2.730 Å for (a). Red: oxygen; yellow: sulfur; blue: nitrogen; aqua: iron; grey: carbon; and white: hydrogen.

Figure 4

a)



b)

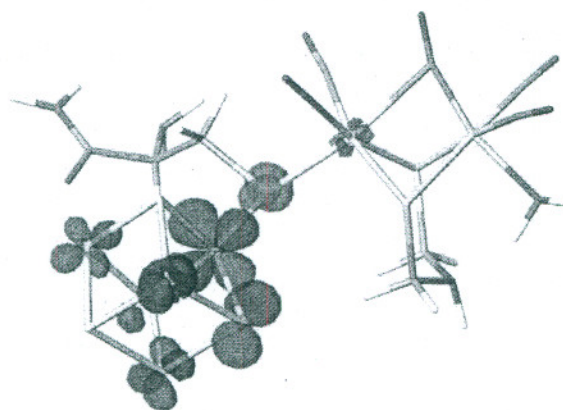
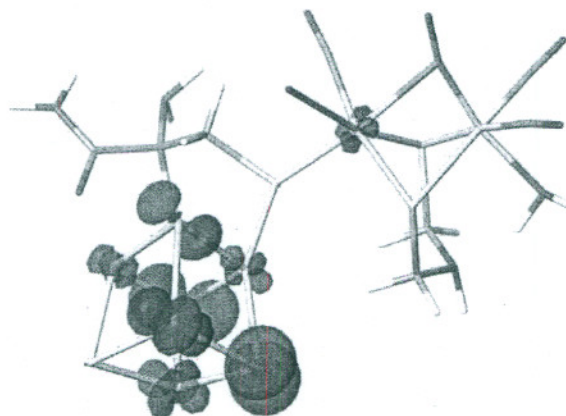


Figure 4

c)



d)

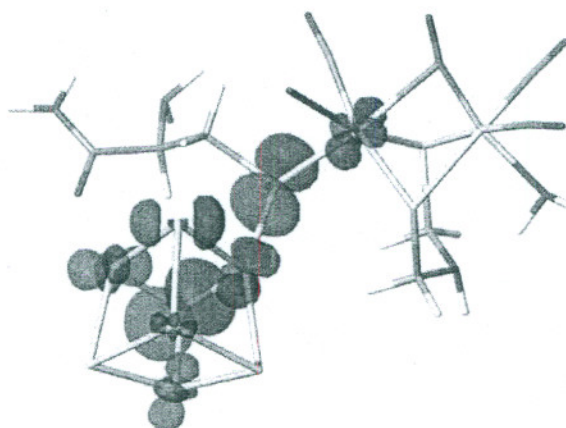
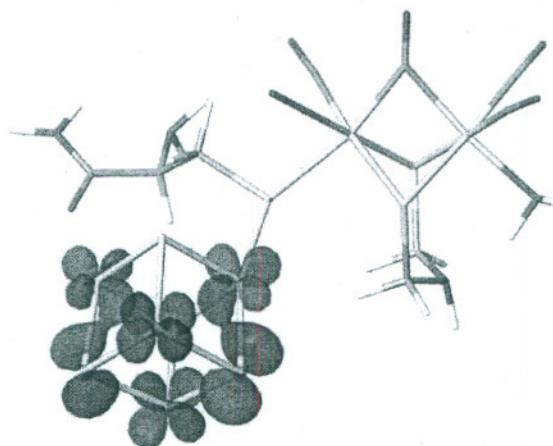


Figure 4

e)



f)

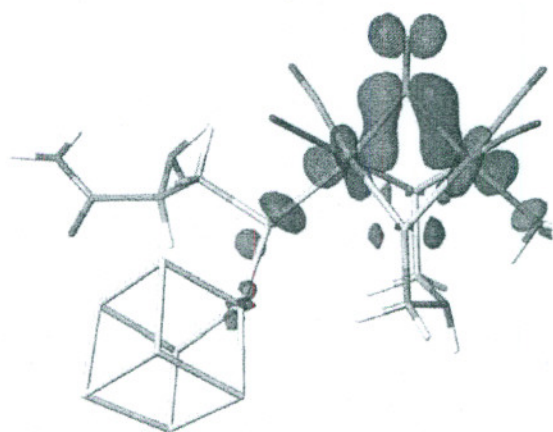


Figure 4

g)

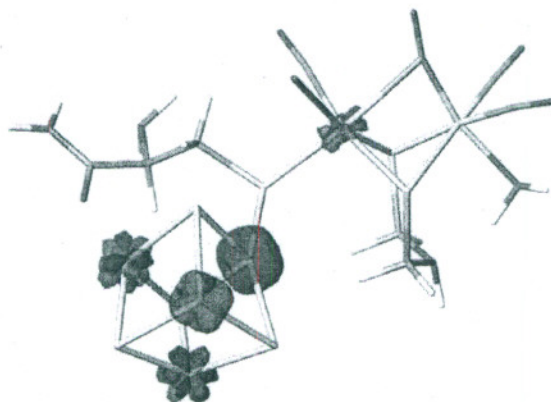


Figure 4. Frontier orbitals and total spin density for model compound C. The HOMO and the LUMO are displayed in (a) and in (b), respectively, for the oxidized form (a molecular charge of +1) of model compound C. The HOMO and LUMO for the partially reduced form (a molecular charge of 0) of C, calculated with unrestricted-spin, are shown in (c) and (d), respectively. The HOMO and LUMO for the reduced form (a molecular charge of -1) are shown in (e) and (f). Total spin density is plotted for partially reduced C in (g). Color legend for atoms is: red: oxygen; yellow: sulfur; blue: nitrogen; aqua: iron; and grey: carbon; and white: hydrogen.

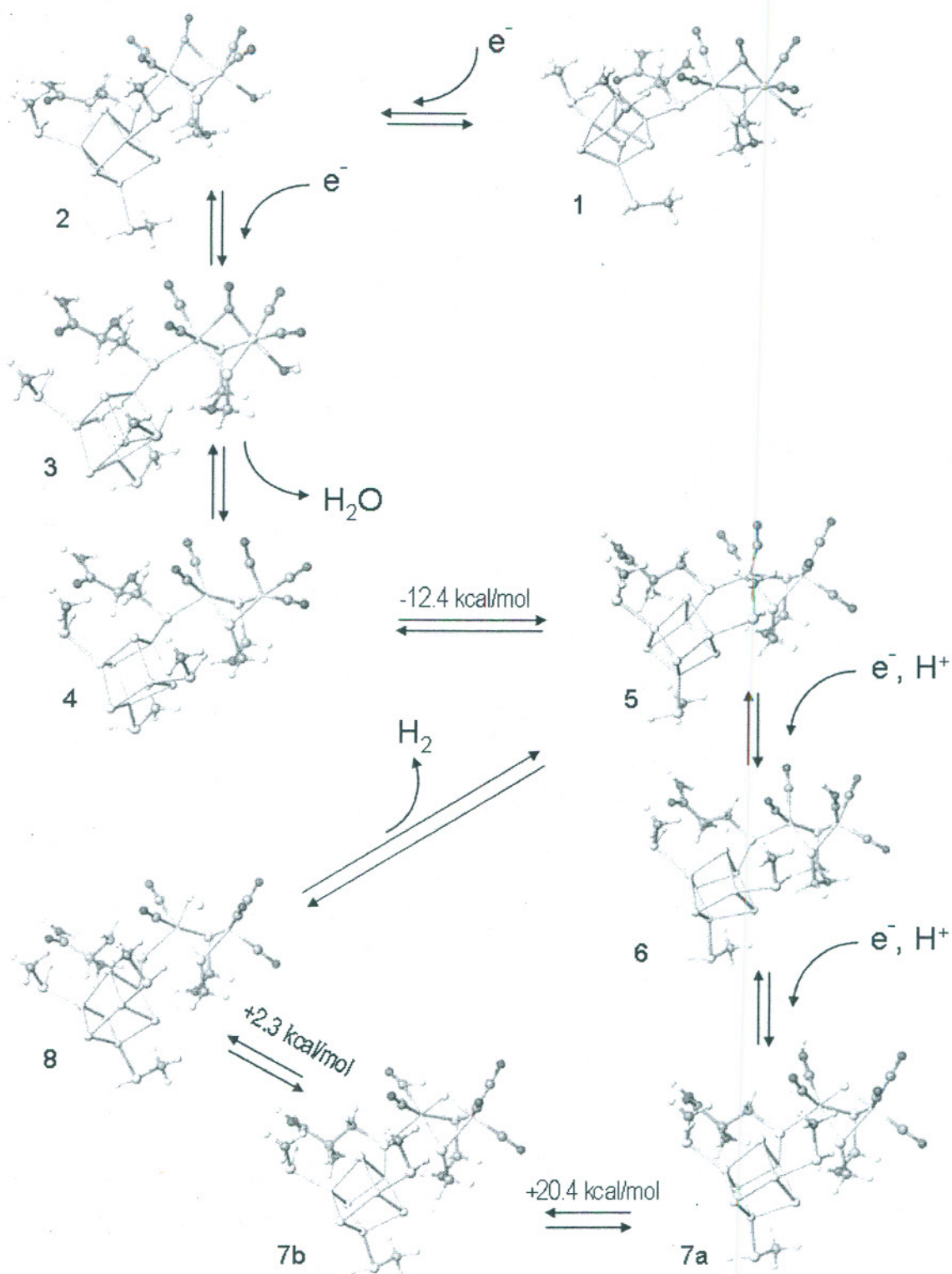


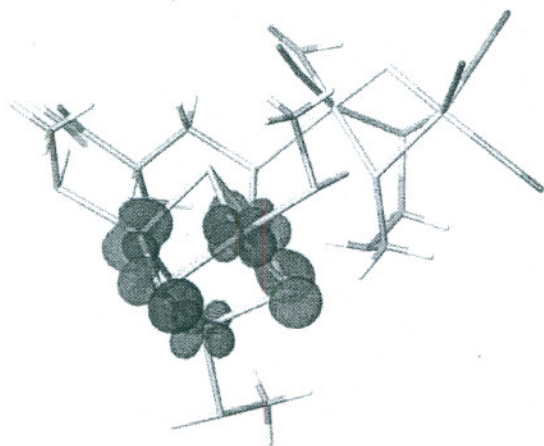
Figure 5. Optimal conformations for 1-8 shown in the same relation to each other as outlined by Armstrong.⁹



Figure 6. Superposition of model compound **8** with the H-cluster from the CpI crystal structure. RMS = 0.740 Å. Only common atoms were used and are shown, as described in the text. Red: oxygen; yellow: sulfur; blue: nitrogen; aqua: iron; and grey: carbon.

Figure 7

a)



b)

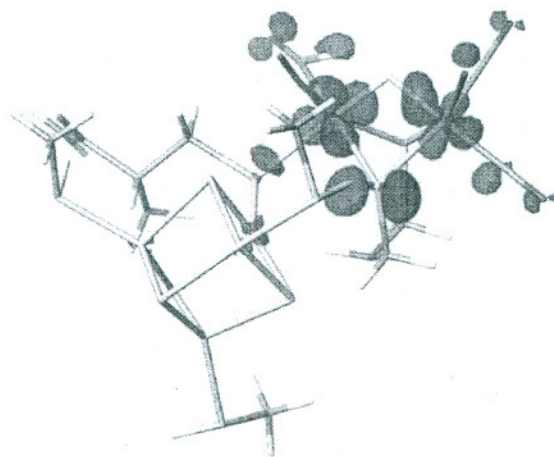


Figure 7

c)



d)

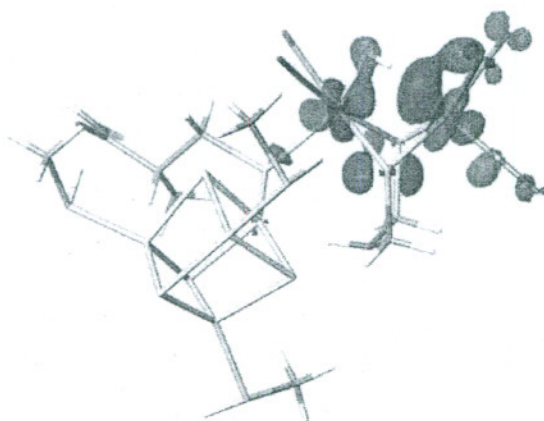


Figure 7. Frontier orbitals for model compounds **7b** and **8**: a) HOMO for **7b**; b) LUMO for **7b**; c) HOMO for **8**; d) LUMO for **8**. Color legend for atoms is: red: oxygen; yellow: sulfur; blue: nitrogen; aqua: iron; and grey: carbon; and white: hydrogen.

Geometry Parameters for model compounds B and C are included as Supporting Information.

Table S1: Geometry Parameters for Model Compound **B**

Bond	CpI ⁴	Oxidized	Semi-Reduced	Reduced	DdH ³
Fe-Fe	2.617 Å	2.558 Å	2.595 Å	2.597 Å	2.566 Å
Fe _p -CN _p	1.857 ^b	1.929	1.941	1.950	1.845
Fe _p -CO _p	1.754	1.798	1.769	1.746	1.712
Fe _p -SCH ₃	2.378	2.360	2.391	2.454	2.505
Fe _d -CN _d	1.863 ^b	1.941	1.942	1.941	1.865
Fe _d -CO _d	1.831	1.805	1.769	1.736	1.797
Fe _d -CO _b	2.043	2.177	1.933	1.763	2.494 ^d
Fe _d -OH ₂	2.959	2.050	3.414	3.741	—
Fe _p -CO _b	2.100	1.883	2.077	2.494	2.807 ^d
Fe _p -S _a	2.319	2.371	2.404	2.399	2.304
Fe _p -S _b	2.340	2.383	2.409	2.417	2.290
Fe _d -S _a	2.337	2.372	2.387	2.413	2.279
Fe _d -S _b	2.320	2.340	2.356	2.385	2.298
Fe _d -C _b -O _b	138°	131°	142°	161°	—
φ _{CO} ^c	178	176	173	-162	174 ^d

^aφ_{CO} is defined in the text.

^bThe CN ligands are assigned as CO ligands in the CpI crystal structure.

^cFor the CpI active site,⁴ the midpoint between the two sulfur atoms was used to define φ_{CO}.

^dThe oxygen atom assigned as the bridging moiety in the DdH crystal structure was used in lieu of CO_b.

Table S2: Geometry Parameters for Model Compound C

Bond	CpI ^a	Oxidized	Semi-Reduced	Reduced	DdH ^d
Fe-Fe	2.617	2.634 Å	2.684 Å	2.542 Å	2.566 Å
Fe _p -CN _p	1.857 ^b	1.937	1.942	1.939	1.845
Fe _p -CO _p	1.754	1.822	1.817	1.806	1.712
Fe _p -S _{cysteine}	2.378	2.261	2.283	2.342	2.505
Fe _d -CN _d	1.863 ^b	1.922	1.927	1.940	1.865
Fe _d -CO _d	1.831	1.848	1.832	1.814	1.797
Fe _d -CO _b	2.043	1.836	1.841	2.066	2.494 ^d
Fe _d -OH ₂	2.959	2.042	2.051	2.044	—
Fe _p -CO _b	2.100	2.438	2.459	1.956	2.807 ^d
Fe _p -S _a	2.319	2.317	2.331	2.352	2.304
Fe _p -S _b	2.340	2.322	2.332	2.370	2.290
Fe _d -S _a	2.337	2.412	2.421	2.376	2.279
Fe _d -S _b	2.320	2.382	2.387	2.346	2.298
Fe _d -C _b -O _b	138°	162°	162°	137°	—
φ _{CO} ^a	178°	175	175	178	174 ^d

^aφ_{CO} is defined in the text.

^bThe CN ligands are assigned as CO ligands in the CpI crystal structure.

^cFor the CpI active site,⁴ the midpoint between the two sulfur atoms was used to define φ_{CO}.

^dThe oxygen atom assigned as the bridging moiety in the DdH crystal structure was used in lieu of CO_b.